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## Extracellular enzyme activity in the mycorrhizospheres of a boreal fire chronosequence

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### ABSTRACT

Saprotrophic microbes are typically credited with producing extracellular enzymes that recycle organic matter, though roots and mycorrhizal fungi also can contribute and may compete with the saprotrophs. We examined extracellular enzyme activity associated with the mycorrhizospheres of arbuscular mycorrhizal, ectomycorrhizal, dual-colonized (arbuscular and ectomycorrhizal), and ericoid mycorrhizal plants in a fire chronosequence in Alaska. Bulk soil and soil from beneath host plants were gathered in July 2004 and assayed for five enzymes that target organic C, P, and N substrates. Compared to bulk soil, activities of the C-targeting enzymes  $\beta$ -1,4-glucosidase and peroxidase were lower in arbuscular mycorrhizospheres and ericoid mycorrhizospheres, respectively. Moreover, extracellular enzyme activity varied among mycorrhizosphere types. Specifically, N-targeting leucine aminopeptidase was highest in arbuscular mycorrhizospheres, followed by ericoid and ectomycorrhizal/dual-colonized mycorrhizospheres;  $\beta$ -1,4-glucosidase had the reverse pattern. In addition, enzymatic stoichiometry suggested that extracellular enzyme producers invested more in C-acquisition than in N-acquisition in recent fire scars compared to mature forests. These data extend previous findings that roots and mycorrhizal fungi compete with saprotrophs by showing that the strength of this effect varies by mycorrhizal host. As a result the community composition of mycorrhizal host plants might mediate enzymatic activity in boreal soils.

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### Introduction

Mycorrhizospheres of plants consist of plant roots and their associated mycorrhizal structures, plus the surrounding soil and microbes directly influenced by them (Rambelli 1973). Numerous studies have established that ecological processes such as decomposition, N mineralization, and microbial community composition can be altered by the presence of plant roots; this phenomenon is referred to as the “rhizosphere effect” (e.g., Hiltner 1904; Katznelson 1946; Cheng et al. 2003). Frequently, roots decrease turnover rates of litter C and increase turnover rates of soil organic C (Cheng and Kuzyakov 2005). Traditionally, mycorrhizal fungi have not been explicitly incorporated within the rhizosphere concept (Timonen and Marschner 2006), even though mycorrhizal fungi colonize the roots of most terrestrial plants (Newman and Reddell 1987; Allen et al. 1995). The most common mycorrhizal groups are arbuscular mycorrhizal fungi, ectomycorrhizal fungi, and

ericoid mycorrhizal fungi. They differ in their morphology, taxonomy, and physiology (Smith and Read 2008), so mycorrhizosphere effects on nutrient cycling could likewise vary depending on the mycorrhizal group involved (Linderman 1988). Nevertheless, few field studies have contrasted mycorrhizosphere effects associated with these three major groups.

Fungi and bacteria conduct nutrient transformations by secreting extracellular enzymes that break down soil organic matter (SOM) and release C, N, and P. Mycorrhizal fungi rely primarily on host plant C, so their contribution to SOM degradation is generally considered modest relative to that of the asymbiotic microbial community (Dighton 2003). However, there is accumulating evidence that mycorrhizal fungi produce a greater variety of enzymes than originally thought, including enzymes that target recalcitrant organic C (reviewed in Cairney and Burke 1998; Burke and Cairney 2002; Read and Perez-Moreno 2003). Thus, mycorrhizal fungi may also directly contribute to SOM decomposition (Talbot et al. 2008).

The extent to which plants and their mycorrhizal fungi contribute to extracellular enzyme production and decomposition in natural systems, however, remains unclear. Plant roots can directly release extracellular phosphatases for P acquisition (Duff

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et al. 1994) and proteases for N acquisition (Godlewski and Adamczyk 2007), but are not generally considered important producers of extracellular enzymes targeting lignocellulosic compounds. Culture-based studies have indicated that the enzyme capacity of mycorrhizal fungi is rarely as high as that of fungal saprotrophs. Specifically, white-rot fungi frequently display higher capacities than ericoid fungi, followed by ectomycorrhizal fungi, and then arbuscular mycorrhizal fungi (Read and Perez-Moreno 2003).

Fires are becoming more frequent in boreal forests, with potential consequences for nutrient dynamics. For instance, annual totals of burned area in Alaska increased 50% in the last decade, ostensibly due to a warmer, drier climate in this region (Kasischke et al. 2010). Fires can alter the nutrient availability of soils, directly via volatilization and mineralization of nutrients, and indirectly via changes in plant community composition and litter quality (Kasischke and Stocks 2000). Specifically, N availability can either increase or decrease following a fire, depending on fire severity and changes in plant community composition; organic material is often burned off and re-accumulates over time; and P availability tends to be greatest in young burn scars (Van Cleve et al. 1983, 1993, 1996; Smith et al. 2000; Treseder et al. 2004; Harden et al. 2006; O'Neill et al. 2006).

These shifts in nutrient availability during ecosystem recovery might alter the nutrient status of plants, mycorrhizal fungi, and other organisms. According to economic principles, plants and mycorrhizal fungi are expected to invest more in the production of a particular extracellular enzyme when the nutrient targeted by that enzyme is limiting to growth (Allison et al. 2010). Furthermore, enzymatic stoichiometry indicates the relative degree to which organisms are investing in acquisition of various nutrients. Previous studies have indicated that ratios of  $\beta$ -1,4-glucosidase:N-acetylglucosaminidase assess acquisition of C versus N (Sinsabaugh et al. 2008, 2009). As ecosystems recover from fire, we might expect that ratios of  $\beta$ -1,4-glucosidase:N-acetylglucosaminidase should be relatively high in fairly young sites, since soil organic C is scarce; and also relatively low where N availability is high. Any shifts as ecosystems recover from fire may have consequences for large-scale nutrient dynamics, since younger ecosystems are becoming more common in the boreal landscape owing to shortening fire-return intervals (Kasischke et al. 2010).

We examined extracellular enzyme activity associated with the mycorrhizospheres of arbuscular mycorrhizal, ectomycorrhizal, and ericoid plants in a fire chronosequence of upland boreal forests of Alaska. Our hypothesis focused on the activities of enzymes in soil associated with the different mycorrhizal plants. Based on laboratory findings (e.g., Read and Perez-Moreno 2003), we hypothesized that enzyme activity would increase in the order of arbuscular mycorrhizal mycorrhizospheres < ectomycorrhizal (or dual-colonized arbuscular mycorrhizal + ectomycorrhizal) mycorrhizospheres < ericoid mycorrhizospheres. In addition, we hypothesized that investment in C versus N acquisition should vary with site age as described above.

## Materials and methods

### Sites

We selected three study areas in upland boreal forests near Delta Junction in the interior of Alaska (63° 55' N, 145° 44' W). All of the sites were on alluvial flats, located within a 100-km<sup>2</sup> area. They represented boreal ecosystems at different successional stages (5, 17, and ~80 years) following severe forest fire. Soils in the sites were predominantly silt loams with underlying deposits of sand and gravel. Permafrost is discontinuous in this region and

was not present in any of these sites. Arbuscular mycorrhizal fungi are most abundant in the younger two sites: arbuscular mycorrhizal hyphal length averages 30–116 km m<sup>-2</sup> in the 5-year old site, 24–134 km m<sup>-2</sup> in the 17-year old site, and 8–16 km m<sup>-2</sup> in the 80-year old site; root length colonized by arbuscular mycorrhizal fungi is 0.69 km m<sup>-2</sup> in the 5-year old site, 0.21 km m<sup>-2</sup> in the 17-year old site, and 0.31 km m<sup>-2</sup> in the oldest site (Treseder et al. 2007). Conversely, ectomycorrhizal fungi are most abundant in the older two sites, with root length colonized by ectomycorrhizal fungi averaging 0.20 km m<sup>-2</sup> in the 5-year old site, 0.51 km m<sup>-2</sup> in the 17-year old site, and 0.30 km m<sup>-2</sup> in the 80-year old site (Treseder et al. 2007). Nitrogen availability (as potential net N mineralization and nitrification) is highest in the 17-year old site (Treseder et al. 2007). Soil organic matter content increases with site age (Harden et al. 2006; Treseder et al. 2007). In this immediate area, P availability (as extractable P) is greater in soils of recently-burned areas than in those of mature forests (O'Neill et al. 2006).

Plant community composition varies among the sites, and standing biomass increases with successional stage (Mack et al. 2008). Herbaceous plants are common at the 5-year old site, particularly arbuscular mycorrhizal-colonized northern rough fescue (*Festuca altaica*). Quaking aspen (*Populus tremuloides*) dominates the canopy of the 17-year old site; this species is dual-colonized by arbuscular mycorrhizal and ectomycorrhizal fungi (Neville et al. 2002). Black spruce (*Picea mariana*), which is colonized by ectomycorrhizal fungi, is the most abundant canopy species at the 80-year old site; ericoid shrubs dominate the understory.

### Sample collection

For each site, we selected the most common arbuscular mycorrhizal host and most common ericoid host in the plots (Table 1). In addition, *Betula glandulosa* was the most common understory ectomycorrhizal host at the 80-year old site, so it was selected. In the two youngest sites, the most common ectomycorrhizal host plant (quaking aspen) was also dual-colonized by arbuscular mycorrhizal fungi. Conversely, no ectomycorrhizal-only host plants were abundant enough to use as study plants in the two youngest sites. Thus, we selected quaking aspen as a representative of a dual-colonized host. The ectomycorrhizal-only mycorrhizospheres did not differ significantly from the dual-colonized rhizospheres in enzyme activities (see Results). Likewise, in no case were plant species within the same mycorrhizal type significantly different from one another (see Results). In other words, the two AM plant hosts used in the study did not significantly differ from one another in extracellular enzyme activities, and neither did the two ericoid plant hosts. To simplify our analyses, we grouped the dual-colonized mycorrhizospheres with the ectomycorrhizal-colonized rhizospheres, and grouped plant species within mycorrhizal type.

Soil samples were gathered in July 2004, at peak growing season. Each plant was partially uprooted, and then soil from the top 15 cm of the mycorrhizosphere immediately within the root system of that plant (determined visually) was transferred to a plastic bag. Roots of other plants could have intermingled with the target plant to some extent, but we expect that the majority of the roots and associated hyphae were from the target plant. The sample was homogenized by hand mixing, placed on ice for transport back to the field laboratory, and then frozen at -20°C. We collected four replicates of each mycorrhizosphere type from each site, for a total of 12 replicates per mycorrhizosphere type. Samples were collected in a blocked design, with four blocks per site, and each mycorrhizosphere type represented within each block. We also collected a sample of bulk soil from each block as a reference. For each bulk soil sample, two soil cores (15 cm deep by 5 cm diameter) were taken randomly within 10 m of one another and compiled to represent bulk soil (four replicates per site). Once frozen, the samples were

**Table 1**

Plant species from which mycorrhizosphere soil was collected.

Mycorrhizal type <sup>a</sup>	5-year old site	17-year old site	80-year old site
Arbuscular	<i>Festuca altaica</i>	<i>Epilobium angustifolium</i>	<i>Festuca altaica</i>
Arbuscular and ectomycorrhizal (dual-colonized)	<i>Populus tremuloides</i>	<i>Populus tremuloides</i>	Not collected
Ectomycorrhizal	Not collected	Not collected	<i>Betula glandulosa</i>
Ericoid	<i>Ledum groenlandicum</i>	<i>Vaccinium uliginosium</i>	<i>Ledum groenlandicum</i>

<sup>a</sup> Mycorrhizal types documented by Allen et al. (1987), Michelsen et al. (1996), Treu et al. (1996), Neville et al. (2002), Cripps and Eddington (2005), and Osmundson et al. (2005).

shipped to University of New Mexico, Albuquerque USA for enzyme analyses. Subsamples of soil were dried at 65 °C to determine moisture content and then combusted at 500 °C to determine organic matter content. All measurements were performed on root-free soil (Sinsabaugh et al. 2005).

### Enzyme analysis

Each soil sample was assayed for two N-acquiring enzymes [N-acetyl-glucosaminidase (which releases glucosamine from chitin and similar polymers) and leucine aminopeptidase (which releases amino acids from protein)], one enzyme that hydrolyzes organic P [acid phosphatase], one enzyme that releases glucose from cellulose [ $\beta$ -1,4-glucosidase] and one enzyme that oxidizes phenolic compounds [peroxidase]. These enzymes are often used as model enzymes representing potential transformation rates of simple (leucine aminopeptidase) versus complex (N-acetyl-glucosaminidase) organic N, a common form of organic P (acid phosphatase), and relatively labile ( $\beta$ -1,4-glucosidase) versus more recalcitrant (peroxidase) organic C (Sinsabaugh et al. 1991). Nevertheless, they cannot represent the entire array of nutrient transformations that could occur in soil. All enzyme assays were conducted using 1.0 g of soil that had been homogenized with 125 ml of sodium acetate buffer (50 mM, pH 5.0), following Sinsabaugh et al. (2005). The N-acetyl-glucosaminidase, leucine aminopeptidase, acid phosphatase and  $\beta$ -1,4-glucosidase assays used methylumbelliferyl (MUB) linked substrates. L-3,4-dihydroxyphenylalanine (DOPA) was the substrate for the peroxidase assay.

The peroxidase assay was conducted in clear 96-well microtiter plates with 16 replicate wells for each sample assay. Eight wells were used as negative substrate controls and an additional eight wells were used for negative sample controls. Assay wells contained 200  $\mu$ l of sample suspension and 50  $\mu$ l of 10 mM DOPA. Negative sample controls contained 200  $\mu$ l sample suspension with 50  $\mu$ l of acetate buffer, and negative substrate control wells contained 200  $\mu$ l of acetate buffer and 50  $\mu$ l DOPA. Ten  $\mu$ l of 3% H<sub>2</sub>O<sub>2</sub> was also added to each well. The plates were placed in an Echotherm incubator at 20 °C for 4 h. Activity was then measured spectrophotometrically at 460 nm using a Molecular Devices VERSAmax plate reader. Following Sinsabaugh et al. (2008), peroxidase activities were calculated as  $\mu$ mol h<sup>-1</sup> g<sup>-1</sup> organic matter of soil, since extracellular enzyme activity tends to covary with organic content of soils.

The N-acetyl-glucosaminidase, leucine aminopeptidase, acid phosphatase and  $\beta$ -1,4-glucosidase assays were conducted on black 96-well microtiter plates, in a similar manner as described above except that eight wells of reference standards and eight wells of quench controls were added for each plate. The reference standard wells contained 200  $\mu$ l of acetate buffer with 50  $\mu$ l of either 10  $\mu$ M 4-methylumbelliferone for acid phosphatase,  $\beta$ -1,4-glucosidase, and N-acetyl-glucosaminidase or 10  $\mu$ M 7-amino-4-methylcoumarin for the leucine aminopeptidase assays. The quench controls contained 200  $\mu$ l of the sample suspension and 50  $\mu$ l of the reference standard. The assays were incubated at

20 °C for 2 h for acid phosphatase,  $\beta$ -1,4-glucosidase, and N-acetyl-glucosaminidase; and 6 h for leucine aminopeptidase. At that time, the reaction was terminated by adding 10  $\mu$ l of 1.0 M NaOH to each well. Fluorescence was measured using a Molecular Devices fmax spectrophotometer set to excitation at 365 nm and emission at 460 nm. Activities were calculated as nmol h<sup>-1</sup> g<sup>-1</sup> organic matter of soil.

### Statistical analysis

To examine changes in enzyme potential among mycorrhizosphere types and sites, we conducted a separate fully-factorial analysis of variance (ANOVAs) for each enzyme. In each case, independent variables were mycorrhizosphere type (including bulk soil) and site. The dependent variable was extracellular enzyme activity or ratios of  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase. In addition, to check for possible differences among species of plant hosts, we conducted ANOVAs with plant species as the sole independent variable. We were unable to transform data sufficiently to meet assumptions of ANOVA, so all analyses were performed on ranked data. Tukey's tests were used for post hoc comparisons. Our first hypothesis, that mycorrhizal plants influence soil enzyme activity, would be supported under significant mycorrhizosphere effects. Our second hypothesis, that enzyme activity would vary between sites, would be supported if  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase ratios differed significantly among sites. Differences are reported as significant when  $P < 0.05$  and marginally significant when  $P < 0.1$ . All statistical analyses were performed using Systat 10 (SPSS 2000).

### Results

Activities of one of the N-targeting enzymes (leucine aminopeptidase) and both C-targeting enzymes ( $\beta$ -1,4-glucosidase and peroxidase) differed significantly among mycorrhizosphere type (Table 2). However, no enzymes followed the hypothesized ranking of arbuscular mycorrhizal < ectomycorrhizal < ericoid. The observation that mycorrhizosphere type did not usually follow expected rankings was contradictory to our hypothesis.

For the two C-targeting enzymes, soil associated with at least one type of mycorrhizosphere displayed lower enzyme activities than did bulk soil (Table 2). Specifically, compared to bulk soil, enzyme production was reduced in the mycorrhizospheres of arbuscular mycorrhizal plants for  $\beta$ -1,4-glucosidase, and in the mycorrhizospheres of ericoid plants for peroxidase. In contrast, N-targeting leucine aminopeptidase activity was highest in the mycorrhizospheres of arbuscular mycorrhizal plants, second-highest in ericoid plants, and lowest in ectomycorrhizal/dual-colonized plants and bulk soil. Activities of N-targeting N-acetyl-glucosaminidase and P-targeting acid phosphatase did not differ significantly among mycorrhizosphere types. However, ratios of  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase, which indicate relative investment in C-acquisition instead of N-acquisition, differed significantly and were highest in bulk soil.

**Table 2**Enzyme activities within mycorrhizosphere types, averaged across sites (means  $\pm$  1 SE).

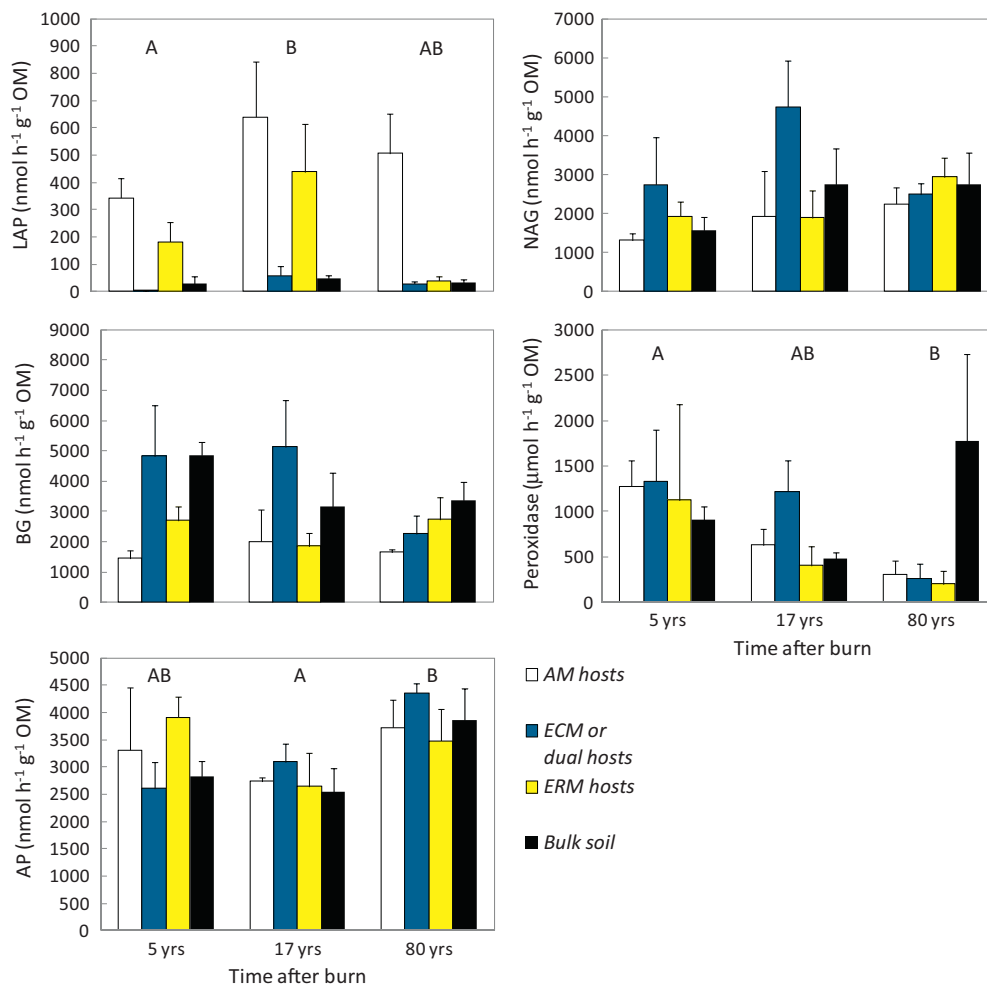
Mycorrhizosphere type	N-targeting		C-targeting		P-targeting	C:N ratio
	Leucine aminopeptidase (nmol h <sup>-1</sup> g <sup>-1</sup> OM <sup>A</sup> )	N-acetyl-glucosaminidase (nmol h <sup>-1</sup> g <sup>-1</sup> OM)	$\beta$ -1,4-glucosidase (nmol h <sup>-1</sup> g <sup>-1</sup> OM)	Peroxidase ( $\mu$ mol h <sup>-1</sup> g <sup>-1</sup> OM)	Acid phosphatase (nmol h <sup>-1</sup> g <sup>-1</sup> OM)	$\beta$ -1,4-Glucosidase:N-acetyl-glucosaminidase
Arbuscular	496 $\pm$ 88 a <sup>B</sup>	1796 $\pm$ 415	1711 $\pm$ 366 a	780 $\pm$ 170 ab	3216 $\pm$ 417	1.07 $\pm$ 0.12 a
Ecto- or dual-colonized	30 $\pm$ 14 bd	3402 $\pm$ 652	4240 $\pm$ 862 b	1001 $\pm$ 264 ab	3261 $\pm$ 299	1.36 $\pm$ 0.16 a
Ericoid	201 $\pm$ 69 c	2288 $\pm$ 307	2490 $\pm$ 328 ab	594 $\pm$ 379 a	3410 $\pm$ 315	1.17 $\pm$ 0.14 a
Bulk soil	37 $\pm$ 10 d	2358 $\pm$ 422	3970 $\pm$ 466 b	1055 $\pm$ 333 b	3075 $\pm$ 287	1.98 $\pm$ 0.33 b
P-value	<0.001	0.221	0.004	0.050	0.790	<0.001

<sup>A</sup> OM = organic matter.<sup>B</sup> Different letters within columns indicate significant pairwise differences at  $P < 0.05$ .

Extracellular enzymes differed in their rankings across sites. In particular, activities of N-targeting leucine aminopeptidase were significantly higher in the 17-year old site than in the 5-year old site, but N-targeting N-acetyl-glucosaminidase did not vary significantly across sites (Fig. 1). Activities of C-targeting peroxidase declined significantly with increasing site age, while C-targeting  $\beta$ -1,4-glucosidase did not change significantly. Phosphorus-targeting acid phosphatase was highest in the oldest site and lowest in the 17-year old site. Site effects were significant for  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase

ratios (Fig. 2); ratios were higher in the 5-year old site than the 80-year old site. The pattern of  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase ratios did not support the second hypothesis. In addition, there was a significant site\*mycorrhizosphere interaction for  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase ratios, driven mainly by particularly high values for bulk soil in the 5-year old site.

It is possible that the pooling of different plant species (and of dual-colonized and ECM-colonized hosts) within mycorrhizosphere type could have led to an overestimation of the significance



**Fig. 1.** Mycorrhizosphere effects on extracellular enzyme activity for N-targeting enzymes leucine aminopeptidase (LAP) and N-acetyl-glucosaminidase (NAG), C-targeting enzymes  $\beta$ -1,4-glucosidase (BG) and peroxidase, and P-targeting enzyme acid phosphatase (AP). Sites differed significantly for leucine aminopeptidase ( $P = 0.029$ ), peroxidase ( $P = 0.038$ ), and acid phosphatase ( $P = 0.015$ ). Values are means  $\pm$  1 SE. Different letters represent significant pairwise differences among sites at  $P < 0.05$ . AM = arbuscular, ERM = ericoid, ECM = ectomycorrhizal, OM = organic matter. Dual hosts were colonized by ectomycorrhizal and arbuscular mycorrhizal fungi.



**Table 3**Enzyme activities within mycorrhizospheres of plant species, averaged across sites (means  $\pm$  1 SE).

Plant species	N-targeting		C-targeting		P-targeting	C:N ratio
	Leucine aminopeptidase (nmol h <sup>-1</sup> g <sup>-1</sup> OM <sup>A</sup> )	N-acetyl-glucosaminidase (nmol h <sup>-1</sup> g <sup>-1</sup> OM)	$\beta$ -1,4-glucosidase (nmol h <sup>-1</sup> g <sup>-1</sup> OM)	Peroxidase ( $\mu$ mol h <sup>-1</sup> g <sup>-1</sup> OM)	Acid phosphatase (nmol h <sup>-1</sup> g <sup>-1</sup> OM)	$\beta$ -1,4-Glucosidase:N-acetyl-glucosaminidase
Arbuscular						
<i>E. angustifolium</i>	640 $\pm$ 202 a <sup>B</sup>	1935 $\pm$ 1146	2007 $\pm$ 1050 ab	634 $\pm$ 177	2740 $\pm$ 75	1.18 $\pm$ 0.14
<i>F. altaica</i>	414 $\pm$ 74 a	1716 $\pm$ 269	1542 $\pm$ 150 b	863 $\pm$ 253	3488 $\pm$ 648	1.00 $\pm$ 0.17
Ectomycorrhizal						
<i>B. glandulosa</i>	29 $\pm$ 7 b	2497 $\pm$ 274	2271 $\pm$ 602 ab	265 $\pm$ 156	4361 $\pm$ 165	0.88 $\pm$ 0.14
Dual-colonized						
<i>P. tremuloides</i>	30 $\pm$ 20 b	3742 $\pm$ 876	4979 $\pm$ 1066 a	1277 $\pm$ 308	2848 $\pm$ 289	1.54 $\pm$ 0.18
Ericoid						
<i>L. groenlandicum</i>	111 $\pm$ 44 ab	2438 $\pm$ 348	2724 $\pm$ 404 a	665 $\pm$ 524	3695 $\pm$ 336	1.20 $\pm$ 0.19
<i>V. uliginosum</i>	439 $\pm$ 175 a	1888 $\pm$ 700	1866 $\pm$ 423 ab	404 $\pm$ 214	2649 $\pm$ 603	1.09 $\pm$ 0.18
P-value	0.001	0.242	0.020	0.138	0.106	0.299

<sup>A</sup> OM = organic matter.<sup>B</sup> Different letters within columns indicate significant pairwise differences at  $P < 0.05$ .

of any site by mycorrhizosphere type interactions. Nevertheless, there were no significant interactions between site and mycorrhizosphere type for any single extracellular enzyme, although there was a marginally significant interaction for peroxidase ( $P = 0.085$ ). Specifically, bulk soil tended to have higher activities than mycorrhizospheres in the 80-year old site, but not in the younger two sites. Dual-colonized (arbuscular mycorrhizal + ectomycorrhizal) mycorrhizospheres (in the 5- and 17-year old sites) did not differ significantly from ectomycorrhizal-only mycorrhizospheres (in the 80-year old site) for any enzyme, based on Tukey pairwise comparisons ( $P > 0.05$  in every case). Plant species displayed significantly different activities of leucine aminopeptidase and  $\beta$ -1,4-glucosidase in their mycorrhizospheres (Table 3). In each case, significant pairwise differences occurred only between plant hosts with different mycorrhizal types.

## Discussion

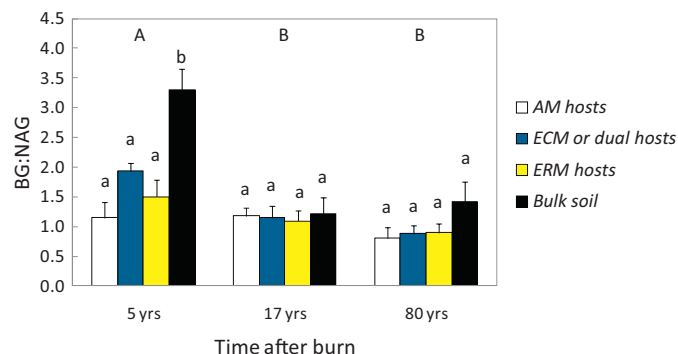
We found that extracellular enzyme activities in boreal soils were not simply a reflection of the expected enzyme capacities of the roots and mycorrhizal groups that dominated a given mycorrhizosphere. Specifically, in no case did enzyme activity significantly increase from bulk soil to arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizospheres, in that order. This

hypothesized order would have occurred if roots and mycorrhizal fungi contribute measurably to soil enzyme production, and if no negative interactions occur between mycorrhizal roots and other soil microbes. Instead, we found that enzyme activities in mycorrhizospheres were sometimes lower than activities in bulk soil, as occurred for  $\beta$ -1,4-glucosidase, which targets cellulose, and peroxidase, which targets phenolic compounds.

These results suggest that negative interactions between saprotrophs and mycorrhizal roots reduced the activity of C-targeting enzymes in mycorrhizospheres compared to bulk soil. This condition could have occurred if roots and mycorrhizal fungi out-competed asymbiotic microbes for nutrients such as N or P, perhaps owing to competitive advantages conferred by availability of plant-provided C. As a result, activities of saprotrophs could have declined where roots and mycorrhizal fungi dominated. If the mycorrhizal roots had lower capacities for production of C-targeting enzymes than did saprotrophs, then activities of these enzymes in mycorrhizospheres would have been reduced. Indeed, laboratory studies have demonstrated that most mycorrhizal groups and plant roots do not produce C-targeting extracellular enzymes to the same extent as many saprotrophs (Duff et al. 1994; Gunther et al. 1998; Burke and Cairney 2002; Read and Perez-Moreno 2003). The differences between bulk soil and mycorrhizospheres were apparent even though bulk soil could have received extracellular enzymes from mycorrhizal fungi colonizing neighboring host plants.

In greenhouse and growth chamber studies, roots frequently elicit a “priming effect” that increases decomposition and microbial activity, ostensibly by augmenting sources of labile C via root exudates and root sloughing (Kuzyakov et al. 2000; Kuzyakov 2002; Cheng and Kuzyakov 2005). These positive effects are especially common in studies measuring the mineralization of SOM (Cheng and Kuzyakov 2005). Nevertheless, inhibition of decomposition by roots can also occur (Cheng and Kuzyakov 2005). Similarly negative effects can be elicited by mycorrhizal fungi (especially ectomycorrhizal fungi), in a phenomenon termed the Gadgil Effect (Gadgil and Gadgil 1971, 1975). The Gadgil Effect has been observed in several field and microcosm studies (Gadgil and Gadgil 1971, 1975; Zhu and Ehrnfeld 1996; Priha et al. 1999), but not always (Staaf 1988; Mayor and Henkel 2006). A mechanism commonly suggested for the Gadgil Effect (or negative root effects) is competition between mycorrhizal fungi (or roots) and saprotrophs for soil resources (Bending 2003; Cheng and Kuzyakov 2005).

Arbuscular mycorrhizal hosts, ectomycorrhizal and dual-colonized plants, and ericoid hosts differed from one another in the enzyme activities associated with their mycorrhizospheres, which implies that the mycorrhizal fungi influenced enzyme



**Fig. 2.** Mycorrhizosphere effects on stoichiometry of extracellular enzyme activity. Ratios of  $\beta$ -1,4-glucosidase to N-acetyl-glucosaminidase (BG:NAG) indicate relative investment in C acquisition versus N acquisition (Sinsabaugh et al. 2008, 2009). Values are means  $\pm$  1 SE. Sites differed significantly ( $P < 0.001$ ). In addition, there was a significant interaction between site and mycorrhizosphere ( $P = 0.004$ ). Different uppercase letters represent significant pairwise differences among sites at  $P < 0.05$ . Lowercase letters represent significant differences among site/mycorrhizosphere combinations.

activities to some extent. Nevertheless, we did not generally observe our hypothesized rankings of highest enzyme activities in rhizospheres of ericoid fungi, followed by ectomycorrhizal and then arbuscular mycorrhizal fungi. Given that competition with saprotrophs may also have been important in these ecosystems, rankings among mycorrhizosphere types could have been influenced by a combination of enzyme capacities of roots, mycorrhizal fungi, and saprotrophs. Specifically, ericoid fungi may be particularly strong competitors with saprotrophs that produce lignin-targeting enzymes, which could contribute to the reduction in peroxidase activities in their mycorrhizospheres. Likewise, ectomycorrhizal fungi may compete only weakly with saprotrophs that degrade cellulose, resulting in a less pronounced reduction of  $\beta$ -1,4-glucosidase activity associated with ectomycorrhizal and dual-colonized mycorrhizospheres compared with the mycorrhizospheres colonized by arbuscular mycorrhizal and ericoid fungi. The mycorrhizospheres of the target plants may have intermingled somewhat with mycorrhizospheres of neighboring plants, so differences between mycorrhizosphere types reported here are likely to be conservative.

Enzymatic stoichiometry can indicate the relative demand for C and N by microbes (Sinsabaugh et al. 2008, 2009). In the study sites,  $\beta$ -1,4-glucosidase:N-acetyl-glucosaminidase ratios were significantly more elevated in bulk soil than in arbuscular mycorrhizal, ectomycorrhizal, and ericoid mycorrhizospheres. Extracellular enzyme activity may have targeted N acquisition (versus C acquisition) more in mycorrhizospheres than in bulk soil. In addition, there was partial support for the second hypothesis regarding C versus N acquisition across chronosequence sites. Specifically, extracellular enzyme producers appear to have invested more in C acquisition (versus N acquisition) in the 5-year old site, given that ratios of activity of  $\beta$ -1,4-glucosidase to N-acetyl-glucosaminidase were relatively high there. Since organic C is relatively scarce in the youngest site, this allocation pattern may mitigate C limitation of microbes to some extent. However, this ratio was not particularly low in the 17-year old site, where N availability is highest; this finding does not support the second hypothesis. Other environmental factors vary across the sites, owing to differences in recovery time following fire. For instance, plant communities shift from herbaceous plants to deciduous trees and shrubs to evergreen conifers as succession proceeds (Mack et al. 2008). In addition, surface soil temperature and soil moisture decline with site age (Treseder et al. 2004). Each of these could have altered the production of extracellular enzymes across sites.

## Conclusions

Our field data are consistent with previous findings in microcosms that roots and mycorrhizal fungi can influence enzyme production by competing with saprotrophs within the mycorrhizosphere. Moreover, enzyme activity varied with mycorrhizosphere type. Only a few previous studies have examined the mycorrhizosphere effect in natural ecosystems, and those based in boreal forests are particularly rare. Given that C-targeting enzyme activities were frequently lower in mycorrhizospheres than in bulk soil, the colonization of soil by plant roots and their mycorrhizal fungi (particularly ericoid and arbuscular mycorrhizal fungi) could potentially slow losses of soil C in boreal ecosystems such as our study sites. In addition, the abundance and community composition of mycorrhizal fungi and their host plants are predicted to shift under global changes such as fires, N deposition, and warming (Rillig et al. 2002). It may be particularly important to consider how interactions between plant roots, mycorrhizal fungi, and saprotrophs can influence nutrient cycling in boreal ecosystems (Leake et al. 2002).

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